

weeks after adoptive transfer, AML in bone marrow (BM) of PR1-CTL treated mice decreased 27% (range 21%-32%; $p < 0.02$) compared to untreated mice, and by 23% (range 4%-47%; $p = \text{NS}$) compared to mice receiving PDC. Moreover, AML in the PB of PR1-CTL treated mice decreased 47% (range 30%-61%, $p < 0.04$) compared to untreated mice, and by 58% (range 48%-68%; $p < 0.01$) compared to mice receiving PDC. These data justify clinical studies to determine whether PR1-CTL isolated directly from UCB can be used to enhance GVL without increased GVHD.

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ANTIGEN PRESENTING CELL-MEDIATED EX VIVO EXPANSION OF HUMAN UMBILICAL CORD BLOOD CELLS YIELDS SIGNIFICANT EXPANSION OF NATURAL KILLER CELLS WITH ANTI-MYELOMA ACTIVITY

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Background: Allogeneic natural killer (NK) cells are active in various hematologic malignancies and may have an important role in multiple myeloma (MM). Umbilical cord blood (UCB) is a potential source for allogeneic NK cells and ex vivo expanded UCB-derived NK (UCB-NK) cells demonstrate activity comparable to that of peripheral blood-derived NK cells. Here we demonstrate the ability to expand fresh and frozen UCB-NK cells to clinical grade by novel techniques using artificial antigen presenting feeder cells modified to express IL-21 ("K562-cl9-mIL21") or IL-15 ("K562-mb-15-41BBL"). These UCB-NK cells demonstrate appropriate phenotype and are active against a variety of MM cell lines.

Methods: UCB-NK cells were expanded from 1) fresh or 2) frozen cord blood units. 1) Fresh cord blood mononuclear cells (CBMCs) were cultured in 10% human serum albumin media with IL-2 (500 IU/ml) and irradiated K562-cl9-mIL21 feeder cells (2:1 feeder: CBMC ratio) for 21 days. Thereafter, cells were subjected to CD3-immunomagnetic depletion. 2) Frozen CBMCs were grown in a gas permeable culture flask with IL-2 (100 IU/ml) and irradiated K562-mb-15-41BBL feeder cells (2:1 feeder: CBMC ratio). Cells were CD3-depleted on day 7, replated with the same conditions and grown until day 14. CD3-negative cells were then used as effector cells in functional assays. Flow cytometry was used to confirm NK cell purity (CD56+/CD3- cells) and a standard chromium-51 assay was performed to determine NK cell cytotoxicity. Targets included K562 cells and MM cell lines RPMI 8226, ARP-1 and U266.

Results: Expansion of fresh and frozen CBMCs yielded a > 2000 and > 200 fold expansion of NK cells, respectively, compared with only 47 fold expansion of fresh CD56-selected cells cultured with IL-2 alone. After CD3 depletion, fresh and frozen-derived UCB-NK cultures were comprised of 92% and 94% CD56+/CD3- cells respectively. APC-expanded UCB-NK cells from fresh and frozen cords demonstrated cytotoxicity against the classic NK cell target K562 as well as MM cell lines RPMI 8226, ARP-1 and U266.

Conclusions: UCB-NK cells can be expanded ex vivo to clinically relevant doses for allogeneic NK cell therapy via co-culture with K562-cl9-mIL21 and K562-mb-15-41BBL feeder cells. Expanded UCB-NK cells are cytotoxic to K562 cells and various myeloma cell lines. Further study of fresh and frozen-derived UCB-NK cells as an adjunct therapy in stem cell transplantation for myeloma is warranted.

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TRANSFER OF SPECIFIC CELLULAR AND HUMORAL ANTI-TUMOR IMMUNITY AFTER STEM CELL TRANSPLANT (SCT) BY VACCINATING STAGE IV BREAST CANCER PATIENTS WITH Her2/Neu TARGETED T CELLS AND TRANSFERRING IMMUNE T CELLS INTO THE PATIENTS AFTER SCT

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Women with stage IV metastatic breast cancer (BrCa) have limited treatment options. Innovative treatment strategies are needed to improve anti-tumor responses. We have recently shown that specific T cell immunity can be induced in patients by "vaccinating" the patients (pts) with infusions of anti-CD3 \times anti-Her2/neu bispecific antibody (Her2Bi) armed activated T cells (aATC). This study investigated whether memory cytotoxic T lymphocytes (CTL) and antibody (Ab) directed at BrCa antigens can be transferred into patients after SCT by evaluating the cytotoxic and Ab responses after aATC infusions prior to SCT and after SCT. After infusions of aATC, peripheral blood lymphocytes (PBL) exhibited high levels of cytotoxicity directed at SK-BR-3 breast cancer cells and high serum levels of Th1 cytokines and IL-12. Three weeks after aATC infusions, ATC were expanded from the second leukopheresis and cryopreserved for re-infusion after SCT. The expanded ATC from 6 patients at an E:T ratio of 25:1 exhibited cytotoxicity ranging from 3.7-25.8 (mean 13.6%) directed at the SK-BR-3. After SCT, pts received multiple infusions with a mean total of 54×10^9 ATC (range 16-110 $\times 10^9$). Cytotoxicity ranged from 4.7 to 70 % from 2 weeks to 12 months post SCT. We tested PBL for the transfer of humoral immunity after SCT by co-culturing PBL with or without SK-BR-3 tumor cells before IT, mid-IT, 1 month post-IT, pre and post SCT for *in vitro* specific anti-SK-BR-3 Ab synthesis. Anti-SK-BR-3 Abs detected in the culture supernatants by ELISA ranged from 15 to 40 ng/ml of Abs prior to IT, 150-220 ng/ml mid-IT and remained high between 70-95 ng/ml at 1 month post-IT. PBL after SCT showed gradual increases in *in vitro* Ab synthesis ranging from 0-10 ng at 1-month post SCT to 70-90 ng by six-month post SCT upon stimulation with tumor cells. Serum anti-tumor Ab levels directed at SK-BR-3 increased from 2-4 μg to 10-12 μg post IT and between 6-9 μg after SCT. No dose-limiting toxicities, delays in engraftment, and life-threatening infections were observed. These data show that transfer of pre-immunized ATC and T cells in the stem cell product enhance tumor specific cytotoxicity after HDC and PBSCT for breast cancer. Data suggest that both cellular and Ab responses can be transferred and provide an anti-tumor immune response early after SCT. This "vaccinate" and "boost" proof-of-principal strategy may be used to design protocols to enhance anti-tumor activity.

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IMPROVED POST-THAW STABILITY VALIDATION OF PERIPHERAL BLOOD CELL PRODUCTS UTILIZING THE INTRACELLULAR-LIKE CryoStor CRYOPRESERVATION SOLUTION, AND PRELIMINARY RESULTS OF CLINICAL APPLICATION

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Collection and cryopreservation of autologous stem cells is a routine procedure in a variety of malignant diseases. Further, improved stability of cell products is critical to the development of cell and tissue based therapies as part of the growth in regenerative medicine. A growing body of evidence indicates that one key method for improved cryopreservation efficacy is the utilization of a hyperosmotic/intracellular-like cryopreservation media, as opposed to the traditional use of an isotonic/extracellular-like media such as saline as the vehicle for the cryoprotectant. In this evaluation of cryopreservation methods for clinical application, cell samples from apheresis products were cryopreserved in a conventional isotonic-based freeze media (CFM; 20% DMSO, 10% human plasma derivative in Ringer solution) or intracellular-like CryoStor CS10 (CS10; 10% DMSO, serum-free and protein-free). Immediately after thawing, the recovery of WBC was $50.7 \pm 14.4\%$ for CFM versus $70 \pm 11.6\%$ for CS10 ($p < 0.001$), and that of CD34+ cells $81.8 \pm 36.1\%$ for CFM and $101 \pm 16.4\%$ for CS10 ($p < 0.05$). In CFM, 20 to 60 min after thawing there was a dramatic loss in cell viability ($\sim 40\%$ to $\sim 90\%$), up to complete clotting in 3/10 samples. By contrast, cells remained viable up to 60 min after thawing in CS10, and no clotting occurred. Because of these positive validation results, cryopreservation in the intracellular-like CryoStor CS10 was translated to clinical application for treatment of hematological malignancies. To date, seven patients have received autologous stem cells cryopreserved in CS10.